

## Claims:

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1. A method for obtaining a soluble protein having a free cysteine comprising the step of:
    - (a) obtaining a host cell capable of expressing the soluble protein;
    - (b) exposing the host cell to a cysteine blocking agent; and
    - (c) isolating the soluble protein from the host cell.
  2. The method of claim 1, further comprising disrupting the host cell in the presence of the cysteine blocking agent and isolating the protein from the soluble fraction of the disrupted host cell.
  - 10 3. The method of claim 1, wherein exposing the host cell to a cysteine blocking agent occurs before, during or after synthesis of the soluble protein by the host cell and wherein the soluble protein is secreted from the host cell.

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    4. The method of claims 1 to 3, wherein said host cell is a bacteria, yeast, insect or mammalian cell.
    5. The method of claims 1 to 3, wherein said host cell is a bacteria cell.
    - 20 6. The method of claim 5, wherein said host cell is *E.coli*.

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      7. The method of claims 1 to 3, wherein said soluble protein is a recombinant protein.
      8. The method of claim 7, wherein said recombinant protein is a cysteine mutein of a member of the growth hormone supergene family, a derivative or an antagonist thereof.
      - 25 9. The method of claim 8, wherein said member is growth hormone.
      10. The method of claim 8, wherein said member is erythropoietin.
      - 30 11. The method of claim 8, wherein said interferon is alpha interferon alpha (IFN- $\alpha$ ).
      12. The method of claim 8, wherein said alpha interferon is interferon alpha 2 (IFN- $\alpha$ 2).
      - 35 13. The method of claim 7, wherein said recombinant protein is a cysteine mutein of a member of the TGF-beta superfamily, platelet derived growth factor-A, platelet derived growth factor-B, nerve growth factor, brain derived neurotrophic factor, neurotrophin-3, neurotrophin-4, vascular endothelial growth factor, or a derivative or an antagonist thereof.

14. The method of claim 7, wherein said recombinant protein is a cysteine mutein of a heavy or light chain of an immunoglobulin or a derivative thereof.

15. The method of claim 1, wherein said cysteine blocking agent is a thiol-reactive compound.

16. The method of claim 15, wherein said thiol-reactive compound is cystine, cystamine, dithioglycolic acid, oxidized glutathione, iodine, hydrogen peroxide, dihydroascorbic acid, tetrathionate, O-iodosobenzoate or oxygen in the presence of a metal ion.

17. The method of claim 15, wherein said thiol-reactive compound is cystine.

18. The method of claims 1 to 3, further comprising attaching a cysteine-reactive moiety to said isolated protein to form a cysteine modified protein.

19. The method of claims 1 to 3, further comprising attaching a polyethylene glycol to said isolated protein to form a pegylated protein.

20. A pegylated human growth hormone (hGH) or a derivative thereof having an  $EC_{50}$  of less than about 110 ng/ml.

21. The pegylated hGH of claim 20, wherein the PEG moiety is attached to a C-D loop or a region proximal to Helix A of said hGH.

22. A pegylated erythropoietin (EPO) or a derivative thereof having an  $EC_{50}$  of less than about 1000 ng/ml.

23. The pegylated EPO of claim 22, wherein the PEG moiety is attached to a C-D loop or an A-B loop of EPO.

24. A pegylated alpha interferon (IFN- $\alpha$ 2) or a derivative having an  $EC_{50}$  of less than about 100 pg/ml.

25. The pegylated IFN- $\alpha$ 2 of claim 24, wherein the PEG moiety is attached to a region proximal to Helix A or a C-D loop of IFN- $\alpha$ 2.

26. A multimeric protein comprising at least two proteins each having a free cysteine and wherein said proteins are attached through said free cysteines.

27. A method of treating a condition treatable with growth hormone, EPO or an alpha interferon, comprising administering to a patient in need thereof an effective amount of a cysteine variant of growth hormone, EPO or alpha interferon, or a derivative thereof to treat said condition.

5 28. The method of claim 27, wherein said derivative is a pegylated cysteine variant of growth hormone, EPO or alpha interferon.

Sub A5 7 29. A method for covalently modifying a protein produced according to claims 1 to 3, comprising the steps of:

- 10 (a) purifying the soluble protein;  
(b) reducing the protein with a disulfide-reducing agent; and  
(c) exposing the protein to a cysteine-reactive moiety to obtain a cysteine-modified protein.

15 30. The method of claim 29, further comprising isolating the cysteine-modified protein from the unmodified protein.

31. The method of claim 29, wherein the cysteine-reactive moiety is a polyethylene glycol.